REMARKS

Claims 24 and 26-37 were examined in the Office Action dated July 8, 2002 and rejected under 35 U.S.C. § 103(a). This rejection is traversed for reasons discussed below. Applicant notes with appreciation the withdrawal of the previous rejection under 35 U.S.C. §102.

Overview of the Above Amendments:

Claims 24, 26, 32 and 35 have been amended to recite the invention with greater particularity. Specifically, the amended claims now recite that the mammalian cell is transduced "*in vivo*" and that ecdysone (in the case of claims 24, 32 and 35) or ponasterone A (in the case of claim 26) is provided to the mammalian cell "*in vivo*." Support for the these amendments can be found throughout the specification, for example, at page 15, line 7; page 27, lines 8-9 and lines 23-26; and Example 5 at beginning at page 36.

Rejections Over the Art:

Claims 24 and 26-37 were rejected under 35 U.S.C. §103(a) as unpatentable over "Versatile Vectors for Ponasterone A- Inducible Control of Gene Expression in Mammalian Cells" from the Stratagene Newsletter, Vol. 12, No.1, first quarter 1999 ("Stratagene") in view U.S. Patent No. 6,117,680 to Natesan ("Natesan-1") and U.S. Patent No. 6,015,709 to Natesan ("Natesan-2"). The Office asserts Stratagene "discloses the use of two vectors for inducing gene expression in a mammalian cell..." Office Action, page 4. The Office correctly acknowledges the "vectors taught by Stratagene are not AAV viral vectors" and Stratagene does "not specifically teach the system having three vectors as claimed in instant claims 26-29 and 32-34 nor in cells already having the RXR receptor as in claims 35-37." Office Action, page 4.

Natesan-1 and Natesan-2 are said to "provide motivation and guidance for using AAV vectors and virions for transduction of mammalian cells...including vectors using the ecdysone receptor system instantly claimed" Office Action, page 4. The Office concludes:

> One of ordinary skill in the art would have been motivated to induce gene expression from a vector that is regulated by ecdysone since Stratagene taught that "the complete control mammalian expression system allows tight control of

gene expression in a wide range of mammalian cell types" since the "inducible promoter used in the system is naturally repressed in the absence of the ecdysone analog ponasterone A (ponA)." Natesan provided the motivation to use this system coupled with AAV viral vectors for cell transduction.

Office Action, page 5. However, applicant disagrees with these assertions.

As explained in the previous response, MPEP 2142 provides that in order to establish a *prima facie* case of obviousness, there must a reasonable expectation of success in obtaining the invention. The reasonable expectation of success, must be found in the prior art, not in applicant's disclosure. See, e.g., *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). Applicant submits that the art of record does not provide a reasonable expectation of success for the claimed invention. All of applicant's claims now require transduction *in vivo*. None of the cited art provides an expectation of success using the claimed AAV systems *in vivo*.

In particular, as acknowledged by the Office, Stratagene nowhere even mentions the use of the ecdysone system for gene regulation with AAV-mediated gene delivery. The Office cites Natesan-1 and Natesan-2 for teaching the use of AAV vectors for transduction of cells using the ecdysone induction system. However, neither of Natesan-1 or Natesan-2 exemplify the use of the ecdysone induction system with AAV. In fact, AAV is only mentioned in passing in Natesan-2 and the ecdysone system is discussed only generally in both Natesan-1 and Natesan-2. The examples in both of Natesan-1 and Natesan-2 do not pertain to the ecdysone system and have nothing whatsoever to do with AAV. Moreover, the examples all pertain to in vitro cell systems. One cannot extrapolate from Natesan's in vitro systems, using unrelated delivery mechanisms, to applicant's claimed AAV-mediated delivery methods. There is absolutely no expectation that success in a non-AAV, in vitro system, would lead to success in an AAV-mediated *in vivo* system. Based on these references, there would be no expectation that the multiple AAV vectors required would transduce the same cells *in vivo*. Additionally, even if the cells *in vivo* were successfully transduced with virions containing each of the required AAV vector constructs, there would be no expectation that the EcR and/or RXR sequences would be transcribed and translated at levels sufficient to block the transcription of the polynucleotide of interest in the absence of ecdysone or an ecdysone analog *in vivo*.

Moreover, it is well established in order to be properly citable art, a reference must be enabling, thus placing the allegedly disclosed matter in the possession of the public. *Beckman*

Instruments, Inc. v. LKB Produkter AB, 13 USPQ2d 1301 (Fed. Cir. 1989). The combined references do not provide an enabling disclosure for the use of complex AAV-based systems for the delivery of multiple vector constructs *in vivo* to regulate gene expression. Applicant submits that the Patent Office itself, were it examining the Natesan patents for compliance with 35 U.S.C. §112, first paragraph with respect to claims pertaining to *in vivo* delivery using AAV-mediated systems, would find the references deficient and nonenabled.

Should the Examiner continue to maintain the rejection over the combination of Stratagene with Natesan-1 and Natesan-2, the only conclusion that can be drawn is that the rejection is premised on an impermissible hindsight reconstruction of the invention based on applicant's disclosure. As stated by the Court of Appeals for the Federal Circuit, '[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). See, also, In re Fine, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988): "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." Thus, it is insufficient merely to show that some or all of the elements of the invention are present in the prior art and possess characteristics of the elements of the instant invention. This basis for rejection is therefore improper and should be withdrawn. Therefore, applicant's claimed invention must be viewed as nonobvious over the stated combination.

CONCLUSION

Applicant respectfully submits that the present claims are patentable. If the Examiner notes any further matters which she believes may be resolved by a telephone interview, she is encouraged to contact M. Christina Thomson by telephone at (510) 748-7208 or by fax at (510) 748-7368.

Respectfully submitted,
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Version with markings to show changes made

Claims 24, 26, 32 and 35 have been amended as follows:

24. (Twice amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell *in vivo* with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) and further comprises a coding sequence encoding a retinoid-X-receptor (RXR), wherein said EcR and RXR coding sequences are operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

26. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell *in vivo* with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises five ecdysone-responsive elements positioned upstream of a heat shock protein (Hsp) promoter sequence, wherein the transcriptional promoter region is capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell; (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo*

transcription of said EcR coding sequence in a mammalian cell; and (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription of said RXR coding sequence in the mammalian cell; and

(b) providing ponasterone A to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

32. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell *in vivo* with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

35. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing a mammalian cell *in vivo* comprising a retinoid-X-receptor (RXR) with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element

(EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

Currently Pending Claims

24. (Twice amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell *in vivo* with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) and further comprises a coding sequence encoding a retinoid-X-receptor (RXR), wherein said EcR and RXR coding sequences are operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

26. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell *in vivo* with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises five ecdysone-responsive elements positioned upstream of a heat shock protein (Hsp) promoter sequence, wherein the transcriptional promoter region is capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell; (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription of said EcR coding sequence in a mammalian cell; and (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-

receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription of said RXR coding sequence in the mammalian cell; and

(b) providing ponasterone A to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

27. The method of claim 26, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

28. The method of claim 27, wherein the enhancer sequence is an SP1 enhancer sequence.

29. The method of claim 27, wherein the transcriptional promoter region comprises three SP1 enhancer sequences.

30. (New) The method of claim 24, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

31. (New) The method of claim 30, wherein the enhancer sequence is an SP1 enhancer sequence.

32. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell *in vivo* with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to

control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

33. (New) The method of claim 32, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

34. (New) The method of claim 33, wherein the enhancer sequence is an SP1 enhancer sequence.

35. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing a mammalian cell *in vivo* comprising a retinoid-X-receptor (RXR) with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell *in vivo*, in an amount sufficient to induce expression of the polynucleotide of interest.

36. (New) The method of claim 35, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

37. (New) The method of claim 36, wherein the enhancer sequence is an SP1 enhancer sequence.